Dalhousie University, Faculty of Dentistry

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Special Edition Microbiology

New Era in Dal Research

Dr. Haroun Shah visited our Faculty in December 1990 to participate in the MRC Site visit for his Development Grant application. Dr. Shah will be taking up his position as a Faculty member jointly appointed between Dentistry and Medicine in July 1991. Haroun is one of the top dental microbiologists in the world. It is indeed fortunate for Dalhousie that we have been able to attracted Haroun to join our faculty. Our International reputation in certain areas of research will now be supplemented with an impressive international reputation in microbiology. This edition of The Dental Research News is devoted to some of the current research of Dr. Haroun Shah. A total of 8 abstracts have been accepted for presentation by Dr. Shah's group at the 1991 British Society for Dental Research meeting. These eight abstracts are reproduced in full on pages 2 to 7.

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New Research Era in Canada??

The House of Commons Committee on industry, science and technology, regional and northern development released 31 recommendations to the government on the 12th December 1990. The most important was a **doubling** of budgets over the next three years for, MRC, NSERC and SSHRC. These agencies had a combined total budget of some $734 million in 1990. The aim is to increase this to $1,468 (+ inflation) by 1993. The recommendations form part of a larger national commitment to science and technology, which will push the spending to 1.9% of the GDP by the year 2000 and 2.5% by the year 2005. Currently Canada spends only 1.3% of GDP on research.

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Happy New Year

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Periodontal Pathogens

The appointment of Haroun Shah marks a major breakthrough in the development of biological research in the Faculty of Dentistry. An indication of the area of research which is currently being pursued by Dr. Haroun Shah's research group can be gained from the following eight abstracts. These abstracts have been submitted and accepted for the British Society for Dental Research Meeting 1991.

1) P. A. Lawson, S. E Gharbia, H. N. Shah & M. D. Collins*.

Strategies studying periodontal pathogens: (1) 16S rRNA sequence analysis.

It is now universally recognised that comparative analysis of 16S ribosomal RNA sequences currently represents the most powerful method for investigating the natural interrelationships of microorganisms and is also of considerable value for constructing nucleic acid probes. In the present study we have therefore examined the partial 16S rRNA primary sequences of members of the genus *Fusobacterium*, *Porphyromonas*, *Treponema*, *Prevotella*, *Tissierella*, *Leptotrichia*, and *Bacteriodes* by reverse transcription. The sequencing strategy generated a continuous stretch of about 1350 bases for each organism. Novel deletions defined each genus. For example, Fusobacterium were characterised by a 22 base deletion in the V6 region of the molecule whereas Treponema possessed major modifications prior to the V3 region of the small subunit ribosomal RNA.

The present study revealed considerable inter- and intrageneric heterogeneity among periodontal pathogens. At the species level taxa were characterised by unique oligonucleotide signatures.

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2) S. E. Gharbia*, H. N. Shah and P. A. Lawson.

Strategies for studying periodontal pathogens: (2) Physiology and Metabolism.

An appreciation of microbial activity of subgingival sites necessitates detailed physiological and metabolic studies of the resident flora. We have undertaken such studies on the key periodontal pathogens *P. gingivalis*, *F. nucleatum* and *T. denticola* using radiolabelled substrates, enzyme assays, transport studies, conductivity measurements, electron transport studies and other methods. All species utilised primarily nitrogenous substrates. In general acidic and basic amino acids were preferentially taken up but their biological function

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varied. For example, *P. gingivalis* utilized glutamate for biosynthetic reactions whereas *F. nucleatum* catabolized this amino acid for energy assimilation. Both *P. gingivalis and T. denticola* possessed electron transport systems but the latter had the capacity to biosynthesize all its precursors. Labelled uptake studies and conductivity measurements revealed a higher affinity for peptides compared to amino acids, however, both their molecular weight and composition varied among species.

These results indicate that periodontal pathogens utilize nitrogenous substrates for energy but differ markedly in both their catabolic amino acids and corresponding anabolic metabolism.

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3) H. N. Shah*, S. E. Gharbia and P. A. Lawson:

Strategies for studying periodontal pathogens: (3) Microbial Interactions.

Our preliminary observations that the combined hydrolytic activity towards diazo-labelled proteins by *P. gingivalis* and *F. nucleatum* increased by >30% prompted detailed studies on microbial interactions among key periodontal pathogens. Such investigations require initial detailed metabolic studies of key fermentable substrates, which were undertaken using a variety of biochemical techniques. Two major proteinases, the cysteine proteinase (gingivain) and a cathepsin-like enzyme of *T. denticola* were potent hydrolytic enzymes against favoured protein hydrolysates such as proteose peptone, trypticase or proteins of crevicular fluid. In all cases, peptides released had growth promoting effects on both the parent species and other organisms that colonize the same ecosystem. Many of these reactions were regulated by the metabolic activity of another species. For example, the reduction required for maximum activity of gingivain (from *P. gingivalis*) was met by the release of low Mr mercaptans from the catabolism of cysteine by *F. nucleatum*.

Results obtained so far indicate that various interactions occur among periodontal pathogens which may have a direct bearing on the process of disease development in subgingival sites.

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Do you have any **"RESEARCH NEWS ITEMS"** which you would like to share with your colleagues.? If so, please forward such items to the Research Development Office, or call 1675.

4) P. A. Lawson*, S. E. Gharbia and H. N. Shah

Ribosomal RNA sequence analysis of *Fusobacterium* species; phylogeny and clinical implications.

The phylogenetic inter-relationship of members of the genus *Fusobacterium* was investigated by comparative analysis of their 16S-ribosomal RNA sequences. The sequence data revealed considerable intrageneric heterogeneity. The four species *F. nucleatum*, *F. alocis*, *F. periodonticum* and *F. simiae* which colonize the oral cavity, exhibited high inter species sequence homology and formed a coherent group quite distinct from the cluster of species, *F. mortiferum*, *F. varium* and *F. ulcerans* which normally reside in the colon. Other fusobacteria displayed no specific relationship. The key periodontal pathogen, *F. nucleatum* was genetically the most diverse and contained four sub species *nucleatum*, subsp. *polymorphum*, subsp. *fusiforme* and subsp. *animalis*. Studies on the distribution and frequency of this species revealed that 82% of subsp. *nucleatum* were isolated from diseased sites whereas subsp. *polymorphum* was only recovered from healthy sites. Only 6% of isolates were subsp. *fusiform*.

Despite the high mol % G+C of some Fusobacterium sp. from the oral cavity (eg. F. alocis, 39%), they form a phylogenetically more coherent group than colonic species. Considerable inter- and intra-species diversity exist. This study revealed, for the first time, that in relation to oral diseases, subsp. nucleatum is clinically the most important member of this group of organisms.

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5) H. N. Shah, S. E. Gharbia and M. J. Kelly*.

Location and functional interaction of the cysteine proteinase and the haemagglutinin of *Porphyromonas gingivalis*.

The haemagglutin (HA) and cysteine proteinase (gingivain) of *P. gingivalis* are considered important virulence factors. We examined their interaction using ^{51}Cr released and electron microscopy. HA was not accompanied by simultaneous lysis, but instead a slow process of attachment and specific attack on the surface of the red blood cells (rbc), leading to the production of minute pores and eventually leakage of its cellular contents. Antisera against the HA of *P. gingivalis* 381 inhibited the HA activity of strain W83 cells, vesicles and supernatant. The

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neutralized supernatant (1:1600) lost 70-80% of BAPNA activity but the residual activity behaved similar to the native supernatant in that stoichiometric amounts of 2,2'-dipyridyl disulphide completely inhibited proteolytic activity which was fully restored upon addition of a low Mr mercaptan. Binding of the antiserum to the HA receptor of *P. gingivalis* still permitted titration of the active centre cysteine of gingivain. Gingivain caused lysis of rbc and was not neutralized by antisera to the HA.

These results suggest that although the HA and gingivain are most likely separate, they are closely sited molecules on the outer membrane of P. gingivalis and may be functionally related.

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6) H. Y. Fraser*, S. E. Gharbia and H. N. Shah

The production of D(-) and L(+)-lactic acid among *Leptotrichia buccalis* strains.

L. buccalis is phylogenetically closely related to *Fusobacterium*, but differs markedly from the latter in producing major levels of lactic acid as a metabolic end product. Apart from its dissolution of enamel, the presence of the D(-) isomer of lactic acid in the blood stream is severely toxic. We have shown previously that a single strain of *L. buccalis* produced mainly D(-) lactate and significantly less L(+) lactate. In the present study, we extend these observations to include 9 clinical isolates and the type strain of *L. buccalis* for their production of lactic acid isomers using enzymatic assays involving D(-) and L(+) lactic acid dehydrogenases (LDH). Lactic acid was produced in the absence or presence of glucose. All but 3 strains produced mainly D(-) lactate (ca 0.6 - 2.65 $g.L^{-1}$) and lower levels of L(+) lactate (ca 0.045 - 1.69 $g.L^{-1}$). Two strains produced > 40 times D(-) than L(+)-lactate. LDH electrophoretic patterns of strains revealed heterogeneity but this did not corroborate with the profiles of strains that produced abnormally high levels of D(-) lactate.

The results of this study revealed heterogeneity in LDH patterns among clinical isolates of L. buccalis. These patterns, however, do not correlate with disparity in D(-) and L(+)-lactate levels among the test strains.

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Justified Research Activity

"no research activity need be justified by its probable contribution to comfort, convenience, or profit." Tyrus Hillway

7) P. A. Lawson, S. E. Gharbia, H. N. Shah and D. R. Clark* :

Dehydrogenases and ribosomal RNA gene restriction patterns among periodontal pathogens.

The application of chemotaxonomic and molecular techniques to the study of periodontal pathogens has resulted in clearer circumscription of many taxa. However, diversity still exists at the species level. Based on a knowledge of the metabolism of these species, we have selected key enzymes to study species diversity based upon their electrophoretic migration. Additionally, we have used ribosomal RNA gene restriction patterns (RFLP), generated by sequence homology to chromosomal DNA of test strains, to study inter species diversity. *P. gingivalis* showed minor variations in malate dehydrogena (MDH) mobilities and was confirmed by RFLP using *Pst* I. *F. nucleatum*, previously shown to comprise 3 groups based on glutamate dehydrogenase and 2-oxoglutarat reductase, was found to comprise further subgroups using *Taq* I and *Eco* RI. *T. denticola* strains were homogenous by MDH and fumarase patterns but heterogeneous by RFLP using *Nci* I.

These studies confirmed that heterogeneity still exists at the species level among periodontal pathogens. The present method is useful for probing the heterogeneity of periodontal pathogens.

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8) S. E. Gharbia, H. N. Shah and R. A. D. Williams*

Growth studies and physiology of oral spirochaetes.

Treponema species represent a significant portion of the colonizing flora of subgingival sites but remain poorly studied due largely to the difficulties of culturing these organisms by conventional laboratory methods. Poor cell yields and growth rates have hampered studies on their physiology. In the present study we have used a variety of modifications of the standard 'NOS' medium to improve the growth yield of species. The best cell yields were obtained with cells cultured on solid 'NOS' media trapped between filter papers (0.22 μm) and glass slides embedded in agar. In liquid media, the best method was 'NOS' containing 0.3% agar utilizing the Hungate System. Attempts to culture cells by tissue culture procedures were highly successful. Monolayers of several cell lines were prepared in tissue culture flasks and the culture fluid replaced with an inoculum of

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the test strains and incubated anaerobically at 37°C. Electron microscopy revealed large numbers of spirochaetes within 4 days attached to specific points of the host cells. Both amino acids and peptides were metabolized but arginine rich peptides were preferentially cleaved.

The growth of oral spirochaetes can be significantly improved by tissue culture methods. Arginine rich peptides were rapidly hydrolysed and represented the favoured growth substrates.

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Empirical Evidence.

"What distinguishes science from the other works of the human imagination is precisely the insistence on testing, on subjecting hypotheses to the most intense scrutiny with the help of empirical evidence. If we are to distinguish science from poetry, we must have a theory of verification or confirmation that tells us exactly how to make that distinction."

Pat Langley *et al.*

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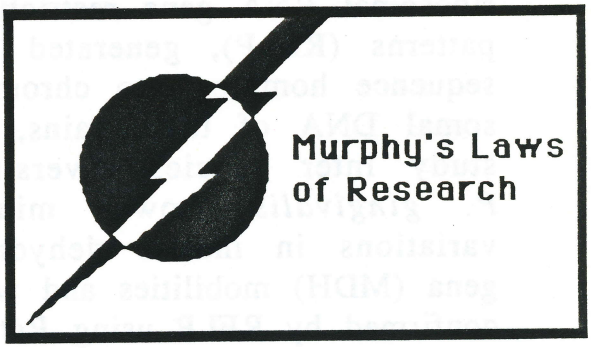
The Final Solution

Famous biologist E. B. Wilson predicted in 1925 that the final solution to every question in biology would be found in the cell. A human being is made up of more than 100 trillion cells, and each is a living unit. Almost every field of science now uses cell culture techniques dentistry has been a bit slow in establishing cell biology laboratories, but that is beginning to change. In our biomaterials research cell culture techniques are well established as a method of evaluating biological effects of compounds being considered for use as biomaterials.

Five or six years ago, there were few cell culture papers presented at the IADR/AADR meetings. At the 1990 IADR session in Cincinnati, cell culture studies accounted for about 13% of all work presented.



18) The number of text books on statistics increases exponentially with the level of your frustration as you try to find a statistical method which will enable you to draw the desired conclusions from your data

The House That Crest Built. The American Dental Association gave Crest tooth paste provisional approval in 1969, and full approval in 1964. By 1964 Crest had about a third of the dentifrice market. A number of the early research clinical trials with fluoride dentifrices failed, this was because the fluoride was inactivated by the abrasive in the toothpaste. It wasn't just a case of adding the fluoride. Three Indiana University researchers Muhler (dentistry) and Day and Nebergall (chemistry) held the patent on the first successful Crest formula, which demonstrated that heat treating the abrasive could make it compatible with fluoride. Indiana University made a contractual agreement with Proctor and Gamble Company to use the patent. Royalties from the sale of Crest were used to build the Preventive Dentistry Research Institute at Indiana often referred to as 'The House That Crest Built.' The patent on Crest expired in 1967 and the institute has never received that level of income since.

When the Institute was dedicated in 1968, it provided an opportunity for all preventive dentistry researchers to be housed under the same roof for the first time. Researchers previously had been scattered in various locations in Bloomington at the medical sciences building, dental and medical schools, and State Board of Health.

In 1972, when Dr. Muhler moved the Preventive Dentistry Research Institute to the Fort Wayne campus, the facility on the IUPUI campus was renamed the Oral Health Research Institute, with Dr. Ralph W. Phillips appointed as director and Dr. Stookey, a 1971 graduate of the PhD program in preventive dentistry, as associate director. Dr. Stookey was named director in 1981.

Today, the Institute is virtually bursting at the seams of its modest-sized quarters (and, in fact, has had to house one of its newest programs across the street at the dental school). The institute which is divided into five sections employs about 50 full-time faculty and staff members; their work is supplemented by nearly 100 individuals who are employed on a temporary basis, depending upon the number and size of the studies being undertaken at any given time.

The pace in our Biomaterials Research Laboratory in swift. (Unless, of course, you count the times when a grant application or abstract deadline is looming up - then, it is **frantic.**

Misinterpreted

"It is important to realize that science does not make assertions about ultimate questions about the riddles of existence, or about man's task in this world. This has often been well understood. But some great scientists, and many lesser ones, have misunderstood the situation. The fact that science cannot make any pronouncement about ethical principles has been misinterpreted as indicating that there are no such principles while in fact the search for truth presupposes ethics."

Karl Popper

Never forget

Concern for man himself and his fate must always form the chief interest of all technical endeavors. Never forget this in the midst of your diagrams and equations. Albert Einstein.

An Age old Problem

"If you thought that cancer was complex, look at aging. If you're are a researcher, you can't go into a better field. We're at the point now that the lay public is recognizing the importance of research in aging, and the funding from the government sector and private sector is increasing."

Edward Schneider

Q) Do frogs have teeth? If so in which jaw?

Answer:

The genus of frog 'Amphignathodon' has teeth in both jaws.

Have You Read

"Public Policy and Human Research," by Albert R. Jonsen, published in Biomedical Ethics Review, edited by James M. Humber and Robert Almeder. Human Press, Clifton, N.J., 1984.

Anuromorpha Research

Did you know that the frog's oral mucosa is enough like the human's to be of possible use in dental research.

Supposing that you conducted an implant research experiment on six birds. The implants in three failed. What type of statistical analysis would you use?